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DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/254407

INTERNATIONAL APPLICATION NO.

PCT/GB97/02477

INTERNATIONAL FILING DATE

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PRIORITY DATE CLAIMED

September 11, 1996

TITLE OF INVENTION

Sulfated Polysaccharides and Uses Thereof in Medical Treatment

APPLICANT(S) FOR DO/EO/US :

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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO (if known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO PCT/GB97/02477		ATTORNEY'S DOCKET NUMBER JJM-399	
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1070.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... \$930.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.455(a)(2)) paid to USPTO..... \$790.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4). \$720.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$98.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	16 - 20 =	0	x \$22.00	\$	
Independent claims	1 - 20 =	0	x \$82.00	\$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 930.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$	
SUBTOTAL =				\$ 930.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$	
TOTAL FEES ENCLOSED =				\$ 930.00	
				Amount to be refunded:	
				charged:	
				\$930.00	

- a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.
- b. ☒ Please charge my Deposit Account No. 10-0750/JJM-399/TJS in the amount of \$930.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 10-0750/JJM-399/TJS. A duplicate copy of this sheet is enclosed.
- NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

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SULFATED POLYSACCHARIDES AND USES THEREOF IN MEDICAL TREATMENT

The present invention relates to novel sulfated polysaccharides obtained by sulfation of cellulose derivatives or polyanionic polysaccharides and uses thereof in pharmaceutical and wound treatment compositions.

Sulfated saccharide and polysaccharide derivatives, in which one or more hydroxyl groups on individual saccharide residues are replaced by sulfate groups, are known. Such sulfated saccharides can occur naturally, as in the case of the sulfated glucosaminoglycans such as heparan sulfate and chondroitin sulfate. Other sulfated saccharides, oligosaccharides and polysaccharides have been prepared by sulfation of naturally occurring saccharides, oligosaccharides or polysaccharides.

A known synthetic sulfated saccharide derivative is sucrose octasulfate, obtained by sulfation of sucrose. The insoluble aluminum salt of sucrose octasulfate (known as sucralfate) has been proposed for a number of medicinal uses, including the enhancement of wound healing in EP-A-0230023.

US-A-2697093 describes the sulfation of cellulose, inulin, starch or dextrin with sulfur trioxide-tertiary amine reagents. The resulting sulfated materials are stated to be useful as thickeners for paste, adhesives, and additives for muds used in drilling oil wells.

Certain sulfated polysaccharides, and in particular the sulfated glucosaminoglycans, are used in medicine as anticoagulants. In addition, heparin, a highly polydisperse copolymer of 1-4 linked glucosamine and uronic acid residues, plays an important role in the control of blood coagulation. Heparin binds the serine protease inhibitor antithrombin producing a complex which accelerates the proteolysis of the enzyme responsible for coagulation. The

anticoagulant properties of heparin and its analogs have been known for some time and have been usefully applied in the biomedical field. The preparation of heparin and sulfated glucosaminoglycans from natural sources is
5 expensive, and therefore an alternative is desirable.

It has now been found that sulfation of cellulose derivatives such as oxidised regenerated cellulose (ORC) or polyanionic polysaccharides such as sodium alginate results
10 in novel sulfated polysaccharide materials that provide unexpected advantages as wound healing materials, and further exhibit anticoagulant properties.

Accordingly, the present invention provides a sulfated
15 polysaccharide selected from a sulfated cellulose derivative and a sulfated polyanionic polysaccharide.

The term "cellulose derivative" encompasses any biologically acceptable ester, ether, hydrolysis product or
20 oxidation product of cellulose. Preferred cellulose derivatives include carboxymethyl cellulose, hydroxyethyl cellulose, cellulose acetate and, especially, oxidized cellulose derivatives such as oxidized regenerated cellulose (ORC), or the oxidized cellulose ethers and esters described
25 and claimed in the provisional US patent application USSN 60/020,758 assigned to the same assignee as the present application and filed on 28th June 1996 and entitled "Bioresorbable Medical Devices from Oxidized Cellulose Derivatives". Preferred materials are obtained by oxidation
30 of hydroxyethyl cellulose, carboxymethyl cellulose or cellulose acetate with dinitrogen tetroxide. The most preferred material is oxidized regenerated cellulose (ORC).

The term "polyanionic polysaccharide" encompasses any
35 biologically acceptable polysaccharide in which a plurality of the saccharide residues in each polysaccharide molecule comprise an anionic group such as carboxylate, or the complementary acid or salt of such an anionic group. It

will be appreciated that some of the cellulose derivatives defined above will also be polyanionic polysaccharides. Preferred polyanionic polysaccharides include alginates, pectins, hyaluronic acid, oxidized starch and oxidized
5 cellulose derivatives as described above. Most preferably, the polyanionic polysaccharide is an alginate. Naturally occurring sulfated polyanionic polysaccharides such as chondroitin sulfate are not included within the scope of the present invention, which relates only to synthetic (i.e.
10 chemically sulfated) polysaccharide derivatives.

Oxidised regenerated cellulose (ORC) is a well known cellulose derivative that is prepared from cellulose as described in US-A-3122479. Oxidation of the primary
15 hydroxyl groups of cellulose to carboxylate groups results in a polyanionic cellulose derivative, namely ORC. ORC is known and used in the biomedical field as a haemostatic agent, as described in US-A-2517772. It is commercially available in the form of a fabric under the registered trade
20 mark Surgicel (Johnson & Johnson Medical, Inc.), and as a haemostatic wound treatment composition under the registered trade mark Interceed (Johnson & Johnson Medical, Inc.). It has been found that ORC is both haemostatic and fully bioabsorbable, and thus provides important advantages as a
25 wound dressing material.

The term "alginate" used herein encompasses alginic acid, soluble salts thereof such as sodium alginate, and insoluble salts thereof such as calcium alginate.
30 Preferably, the alginate is calcium alginate, which is available for use in wound dressings under the Registered Trade Marks Instat (Johnson & Johnson Medical, Inc.) and Kaltostat (E.R. Squibb & Sons, Inc.). Preferably, the alginate has a molecular weight in the range 50,000 to
35 400,000 and a mannuronic acid/guluronic acid ratio of about 0.45.

The sulfated polysaccharides according to the present

invention differ from the non-sulfated base materials in that at least some of the hydroxyl groups on the saccharide residues of the polysaccharides have been converted to sulfate groups. Preferably, an average of at least 0.1
5 hydroxyl groups on each saccharide residue have been converted to sulfate groups. More preferably, at least 1 hydroxyl group, on average, on each saccharide residue has been converted to sulfate groups, and most preferably from 3 to 4 hydroxyl groups on each saccharide residue have been
10 so converted.

The sulfated polysaccharides can exist in the free acid form, but will more normally be used in the form of a salt or hydrate. The salt may be a salt with an alkali metal
15 cation such as sodium or potassium, or may be a salt formed with polyvalent ions such as calcium or aluminum (by analogy with sucralfate), or may be a salt formed with complex cations such as ammonium or alkylammonium. Of course, the sulfated polysaccharide or its soluble salts may be stored
20 or used in the form of a buffered aqueous solution. In any case, the term "sulfated polysaccharide" in the present specification encompasses all such salts and hydrates.

The sulfated polysaccharides according to the present
25 invention can be made with substantially any molecular weight from 500 to 5,000,000, depending on the molecular weight of the starting material. Low molecular weight sulfated polysaccharides generally have alkali metal salts that are soluble in water, and accordingly such materials
30 having molecular weights in the range 1,000 - 50,000 are preferred for some applications. These materials preferably have solubility of at least 10g/l, more preferably at least 25g/l in water at pH7.

35 When an insoluble sulfated material is desired, the base polysaccharide preferably has an average molecular weight in the range 50,000 - 250,000, more preferably 50,000 - 150,000. Such sulfated polysaccharides and/or their salts

are usually substantially insoluble in water. For example, insoluble sulfated ORC can be prepared from commercially available fibrous ORC in the form of a woven, non-woven or knitted fabric, thereby providing a sulfated ORC in the form of a fabric.

The present invention also provides a pharmaceutical composition comprising a sulfated cellulose derivative and/or a sulfated anionic polysaccharide according to the present invention. The term "pharmaceutical composition" includes wound treatment compositions and wound dressings and implants, as well as topical, oral or parenteral medicinal compositions.

It has been found, surprisingly, that the sulfated polysaccharides according to the present invention have an exceptional ability to bind to matrix metalloproteinases (MMP's). Such matrix metalloproteinases are implicated in a number of medical conditions, including chronic wounds such as decubitis ulcers. This is because the balance between matrix deposition and tissue turnover, which in turn may depend on the balance between proteolytic enzymes and their inhibitors, is fundamental to wound healing and to certain other medical conditions. Chronic wound fluids have been shown to contain elevated levels of MMP2 (Gelatinase A) and MMP9 (Gelatinase B).

Accordingly, the present invention further provides the use of a sulfated polysaccharide according to the present invention for the preparation of a composition for the treatment of medical conditions mediated by a matrix metalloproteinase. Preferably, the medical condition is a wound, especially a chronic wound or ulcer.

This aspect of the invention further encompasses a method for the treatment of a chronic wound such as a venous ulcer or a decubitis ulcer in a mammal by the topical or systemic administration of a pharmaceutical composition

containing the sulfated polysaccharide according to the present invention, preferably together with conventional pharmaceutical excipients. Preferably, the sulfated oligosaccharide is applied topically as or in a wound dressing composition. Preferably, the sulfated polysaccharide is applied in an ointment containing 0.1%-10% w/w of the sulfated polysaccharide together with conventional excipients such as water and a gelling agent.

10 It has also been found, surprisingly, that the sulfated polysaccharides according to the present invention has anticoagulant properties. Such properties are the exact opposite of the haemostatic properties well known for ORC itself. Accordingly, the present invention further provides
15 the use of a sulfated polysaccharides according to the present invention for the preparation of an anticoagulant medicinal composition.

This aspect of the invention further comprises a method
20 for the preparation of blood coagulation in a mammal by the topical or systemic administration of a pharmaceutical composition containing the sulfated polysaccharide according to the invention.

25 The pharmaceutical compositions according to the present invention may further comprise conventional pharmaceutical excipients and/or other active agents. They may be adapted for topical, oral or parenteral administration.

30

The sulfated anionic polysaccharides according to the present invention can be prepared by sulfation of a cellulose derivative or a polyanionic polysaccharide with sulfur trioxide-tertiary amine complexes or sulfur trioxide-pyridine complexes in a suitable inert polar solvent, such
35 as N-methyl pyrrolidone. The degree of sulfation can be controlled by adjusting the molar ratio of polysaccharide to sulfur trioxide, since the reaction is essentially

quantitative. The extend of sulfation can be monitored by nmr and elemental analysis, and in particular by Infrared spectroscopy.

5 If a low molecular weight, soluble, sulfated polysaccharide derivative is desired, then the sulfation is preferably carried out on a low molecular weight, soluble oligosaccharide. For example, the sulfation can be carried out on ORC oligosaccharides obtained as described in the
10 present applicant's co-pending United Kingdom patent application no. 9613683.3 entitled "Oxidised Oligosaccharides". Briefly, the ORC oligosaccharides are prepared by treating commercially available ORC with 6M sodium hydroxide solution at 37°C for 45 minutes, followed
15 by filtration. The filtrate containing the ORC oligosaccharides in solution is subjected to neutralisation and dialysis to remove fragments and impurities having molecular weight below 1000, and is then freeze-dried.

20 Specific embodiments of the invention will now be described further, by way of example, with reference to the accompanying drawings, in which:

Figure 1 shows the measured amount of MMP binding for
25 a sulfated ORC derivative according to the present invention, compared with ORC itself and also compared with a collagen control; and

Figure 2 shows the measured amount of MMP2 and MMP9
30 binding for sulfated alginate/collagen sponges compared with a pure collagen sponge and non-sulfated alginate/collagen sponges.

Example 1

35 Milled ORC (Interceed®, 10g) was activated by slurring in 100ml deionised water with stirring for 40 minutes at 40°C. The slurry was filtered using a Buchner apparatus, and the resultant cake was resuspended in 100ml glacial

acetic acid and stirred for a further 30 minutes at 4°C to remove any excess water. The slurry was again filtered and washed extensively with N-methyl pyrrolidone (NMP) until all the acetic acid was removed.

5

Pyridine sulfur trioxide (40g) was dissolved in 40ml N-methyl pyrrolidone, cooled to 4°C, and slowly added to the activated ORC. The slurry was stirred at 4°C for 2 hours, and the pH was then adjusted to 5.6 by the careful addition
10 of cold 2M NaOH. The neutralised slurry was poured into three volumes of methanol and the precipitate collected. The sulfated material was washed with several portions of methanol to remove the N-methyl pyrrolidone before being washed with distilled water to remove unbound sodium
15 sulfate. The material was frozen and freeze-dried.

FT-IR analysis of the material revealed that the all of the primary hydroxyl groups and one or more of the secondary hydroxyl groups had been sulfated. The product is
20 the insoluble sodium salt of the sulfated ORC.

Example 2

The procedure of Example 1 was repeated on a 10g sample of Interceed® ORC fabric, resulting in a sulfated ORC fabric
25 product.

Example 3

A composite material consisting of a complex between
30 the sulfated ORC according to the present invention and collagen was prepared as follows.

Sulfated ORC fibres (0.875g) prepared as in Example 1 were suspended in 10ml distilled 0.06M acetic acid and
35 stirred until fully dispersed. Bovine collagen, which was fully limed, freeze-dried and milled to 1mm fibers (1.625g) was slurried in 250ml 0.05M acetic acid and homogenised for 15 seconds on a Waring Blendor. The suspension of sulfated

ORC was added to the collagen slurry, and the mixture was homogenised for a further 15 seconds. The slurry was cross-linked by the addition of 0.0325ml of HMDI, followed by homogenisation for 2 x 15 seconds. The slurry was degassed
 5 under vacuum, poured into 9cm by 9cm petri dishes, frozen and freeze-dried. The resultant sponges were sterilised with gamma-irradiation.

Example 4

10 A sulfated calcium alginate was prepared from Alginic acid supplied by Sigma Chemical Company (A7003). A sample of this material (10g) was sulfated as described in Example 1.

Example 5

15 A composite material consisting of a complex between the sulfated alginate obtained in Example 4 and collagen was prepared by the procedure described in Example 3, using milled sulfated alginate fibers in place of the sulfated ORC
 20 fibers.

Procedure 1: Effect of Sulfation on Matrix Metalloproteinase Binding by ORC

The effect of sulfation of ORC on matrix
 25 metalloproteinse (MMP) binding was assessed as follows.

Sulfated ORC was prepared by the procedure described in Example 2. An ORC-free collagen sponge was prepared for comparison purposes. A sample of Surgicel® ORC fabric was
 30 also prepared for comparison.

Briefly 50mg of each material was placed in a 15ml plastic beaker containing 2.5ml of an acute wound fluid diluted to 1:50 in a proteolysis buffer (50mM tris/HCL
 35 pH7.8, 50mM CaCl₂, 0.5M NaCl) and incubated at 37°C on a shaking water bath for 3 hours. Acute wound fluid contains various proteinases, including matrix metalloproteinases and many of these enzymes will preferentially bind to various

dressing materials. The excess fluid absorbed by each material was mechanically expressed using a metal spatula and discarded. The remaining dressings were placed into pre-packed 2ml syringes (each syringe contained 0.5ml volume of 2.5mm glass beads). 4ml of proteolysis buffer was forced through the syringe in 1ml aliquots which were discarded. At this washing stage all of the unbound proteinases and proteinaseses which were only weakly bound to the dressing material had been removed from the dressing leaving the more tightly bound forms. The buffer rinsed dressing were then removed to another 15ml plastic beaker. 1ml of non-denaturing sample buffer (6.3ml 0.05M tris/HCL pH6.8, 2.5ml glycerol, 0.5g SDS, 16.2ml water and bromophenol blue) was added to each sample which were placed on an orbital shaker at setting six for 2 hours. The sample buffer detaches the tightly bound proteinases from the materials which are then present in the sample buffer itself. After this time 20 microliters of sample buffer was taken from each container and subjected to gelatin substrate SDS-polyacrylamide gel electrophoresis (zymography) as described by Heussen C. and Dowdle E.B. in Anal. Biochem. 102:196-202 (1980).

The area of the individual zones of clearance on the gels, which are due to proteinase activity, were accurately measured by the Optilab system. This was achieved by repeating each binding experiment (n=3) and analysing the results statistically by the Students T test, where $P \leq 0.05$. Analysis was against controls of pure collagen.

The results shown in Figure 1 demonstrate a surprising synergistic improvement in MMP binding for the sulfated ORC. In Figure 1, Pro2 and Pro9 are the proenzyme forms of MMP2 and MMP9. Both MMP2 and MMP9 can exist as active and proenzyme forms. The molecular weight of the proMMP9 or pro9 is 92kDa. MMP activation is a two step process, a conformational change occurs in the proenzyme, followed by removal of the propeptide by a series of autocatalytic changes. The molecular weight of the resultant active forms

being 86kDa for ActMMP9 and 66kDa for ActMMP2, sometimes called ACT9 and ACT2 for short.

Procedure 2: Effect of Sulfation of Alginate on Matrix

5 Metalloproteinase Binding by Collagen-Alginate Complexes:

Procedure 1 was repeated on the following samples: a pure collagen sponge (comparative experiment), collagen/calcium alginate sponges containing 5% and 20% w/w of alginate (comparative experiments identified as 5% ALG and 20% ALG), and collagen/sulfated calcium alginate sponges containing 5%, 10% and 20% w/w of sulfated calcium alginate, prepared as described in Example 5 and identified as 5% SALG, 10% SALG and 20% SALG.

15 The results are shown in Figure 2. It can be seen that the collagen/sulfated alginate sponges exhibit much stronger binding to both MMP2 (Gelatinase A) and MMP9 (Gelatinase B) than the comparative sponges.

20 Procedure 3: Effect of Sulfation of ORC on Blood Coagulation

The effect of sulfation on the coagulant properties of ORC is studied as follows.

25 Fresh rat's blood (1ml) is measured into 7ml sterile tubes containing 0(control), 0.1g or 1.0g of sulfated ORC fibers prepared as in Example 1. The tubes are gently agitated for 1 minute, and then at 20 second intervals. The length of time taken for the blood to clot is recorded. The results are as follows:

	Weight of Sulfated ORC Fibers (g)	<u>Time to Clot(s)</u>	
		<u>With Sulfated ORC</u>	<u>Control</u>
35	0.1	510	380
	1.0	735	490

40 This demonstrates that the sulfated ORC fibers have an anticoagulant effect. This result is unexpected in view of

the known haemostatic properties of ORC itself.

Procedure 4: Effect of Sulfated ORC on Haemostatis

The effect of sulfated ORC on haemostasis was
5 investigated using the swine spleen model, as follows:

Recently weaned, female, cross-bred swine, in the
approximate weight range of 22-45kg, were anaesthetised with
Isoflurane (Aerrane®). A surgical plan of anaesthesia was
10 achieved and demonstrated by a null response to a noxious
stimulus. While under anaesthesia, physiological parameters
such as temperature, pulse and respiration were monitored
and documented.

15 Animals were placed in dorsal recumbrance with all
limbs secured. The abdominal cavity was opened along the
midline. The spleen was exteriorized. Haemostasis incision
wounds were made using a scalpel on the surface of the
spleen. Wound lengths were controlled and ranged from 0.5
20 to 2.0cm. Wound depth was controlled and ranged from
approximately 1.5 to 3.0mm deep. The depth of each wound
was kept constant by marking the scalpel blade at the
appropriate depth. The length of the incision was
controlled by using a suitable template which had been
25 clearly marked for the appropriate incision length. The
first wound at the distal end of the spleen served as a
negative control and was permitted to bleed for twelve
minutes to demonstrate the bleeding potential of an
untreated wound. The second wound was made approximately
30 1.0cm proximal to the first incision. This and subsequent
incisions were used as test incisions. A final incision was
used as a termination negative control to demonstrate that
the bleeding potential of an untreated wound did not change.

35 After incisions were created, a stop-watch was started
and collagen sponge (Instat) or collagen/sulfated ORC sponge
prepared as in Example 3 were quickly applied to the wounds.
Gentle pressure was applied to the top surface or the gauze

for 2 minutes and then the pressure was released. This procedure was repeated at 30 second intervals until the haemorrhage was controlled. Control of the haemorrhage (haemostasis) was defined as no renewed bleeding for 30
5 seconds. The time of the last release of pressure was the time to achieve haemostasis. The order in which the test or control articles were placed on the wounds was assigned by computerised randomisation.

10 Animals were euthanized by I.V. injection with a commercially available solution or other suitable means before recovering from anaesthesia.

These studies showed that the presence of sulfated ORC
15 retarded haemostasis relative to a pure collagen sponge.

The above described specific embodiments of the present invention are intended solely for the purpose of illustration. Many other embodiments falling within the
20 scope of the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. Use of a sulfated polysaccharide selected from the group consisting of sulfated cellulose derivatives and sulfated polyanionic polysaccharides for the preparation of a composition for the treatment of a chronic wound.
2. Use according to claim 1, wherein the sulfated polysaccharide is selected from the group consisting of sulfated hydroxyethyl cellulose, sulfated carboxymethyl cellulose and sulfated oxidized regenerated cellulose.
3. Use according to claim 2, wherein the sulfated polysaccharide is sulfated oxidized regenerated cellulose.
4. Use according to claim 1, wherein the sulfated polysaccharide is selected from the group consisting of sulfated alginates, sulfated pectins and sulfated hyaluronic acid.
5. Use according to claim 4, wherein the sulfated polysaccharide is a sulfated alginate.
6. Use according to any preceding claim, wherein the sulfated polysaccharide comprises an average of at least 0.1 sulfate groups for each saccharide residue of the polysaccharide.
7. Use according to claim 6, wherein the sulfated polysaccharide comprises an average of at least 1 sulfate group for each saccharide residue of the polysaccharide.
8. Use according to any preceding claim, wherein the sulfated polysaccharide has an average molecular weight in the range 25,000 to 250,000.
9. Use according to any preceding claim, wherein the sulfated polysaccharide is in the form of a woven, non-woven or knitted fabric.

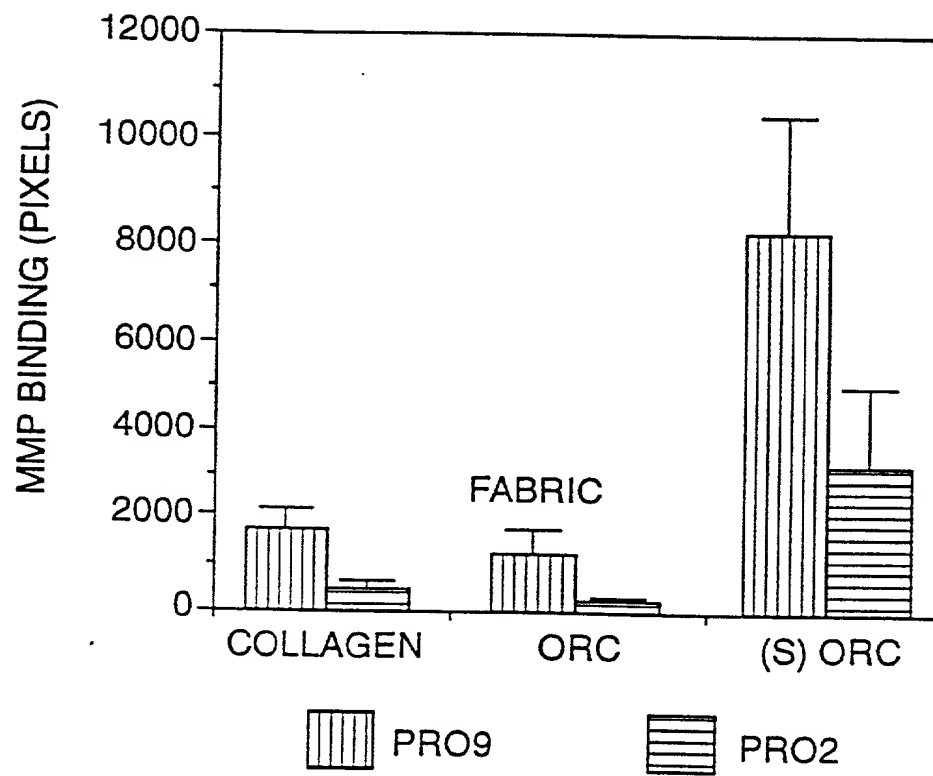
AMENDED SHEET

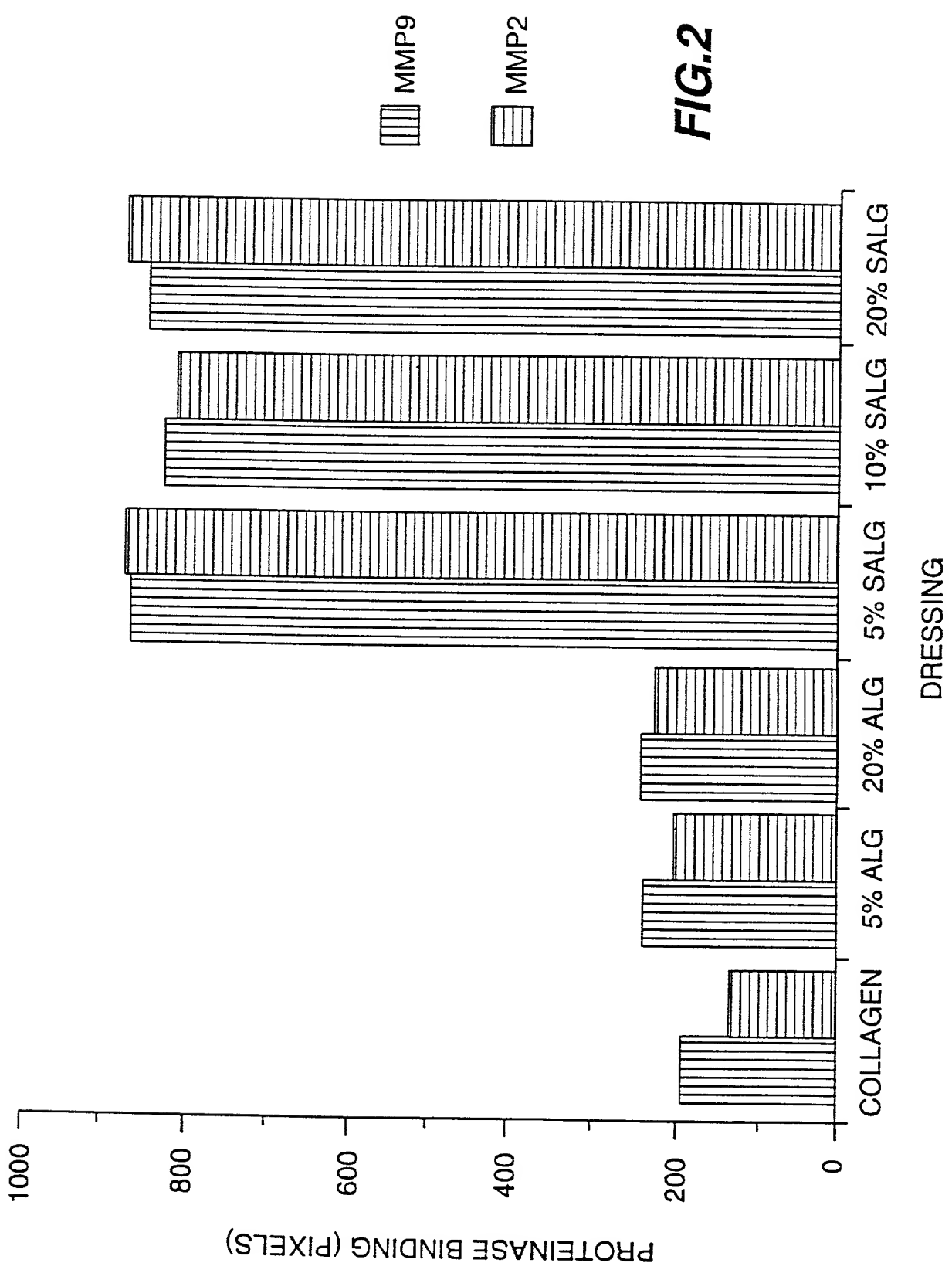
10. Use according to any preceding claim, wherein the sulfated polysaccharide is in the form of a solid complex with collagen.
11. Use according to any preceding claim, wherein the sulfated polysaccharide is
5 soluble in water to an extent of at least 10 g/l at 25°C.

ABSTRACT**SULFATED POLYSACCHARIDES AND USES THEREOF IN MEDICAL TREATMENT**

5 The invention provides sulfated oxidised regenerated
cellulose compounds, sulfated alginates, and the salts and
hydrates thereof. The compounds are obtained by sulfation
of oxidised regenerated cellulose with sulfur trioxide. The
invention also provides pharmaceutical compositions
10 comprising the compounds, in particular compositions for the
treatment of medical conditions mediated by a matrix
metalloproteinases and anticoagulant pharmaceutical
compositions.

15 **(Figure 1)**

FIG. 1





DOCKET NO. JJM-399

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled SULFATED POLYSACCHARIDES AND USES THEREOF IN MEDICAL TREATMENT, the specification of which

(check one) ☐ is attached hereto.

☒ was filed on March 5, 1999 as

Application Serial No. 09/254,407

and was amended on _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.



DOCKET NO. JJM-399

Prior Foreign Application(s):

Country	Application Number	Date of Filing	Priority Claimed Under 35 U.S.C. 119	
Great Britain	9618958.4	September 11, 1996	<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
			<input type="checkbox"/> YES	<input type="checkbox"/> NO
			<input type="checkbox"/> YES	<input type="checkbox"/> NO

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

(Application Number)(Filing Date)(Application Number)(Filing Date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

PCT/GB97/02477
Application Serial No.

September 10, 1997
Filing Date

Pending
Status

Application Serial No.

Filing Date

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1 I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith as well as to file equivalent patent applications in countries foreign to the United States including the filing of international patent applications in accordance with the Patent Cooperation Treaty: Audley A. Ciamporzero, Jr. (Reg. #26,051), Steven P. Berman (Reg. #24,772), Andrea L. Colby (Reg. #30,194), Michael Stark



Docket No: JJM-399

(Reg. #32,495), Joseph F. Shirtz (Reg. #31,880), Andrew C. Farmer (Reg. #35,868), and Theodore J. Shatynski (Reg. #36,676), One Johnson & Johnson Plaza, New Brunswick, NJ 08933.

Address all telephone calls to Theodore J. Shatynski at telephone no. (732) 524-2498.

Address all correspondence to Audley A. Ciamporcerro, Jr., One Johnson & Johnson Plaza, New Brunswick, NJ 08933-7003.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Inventor's Signature:
Full Name of Sole
or First Inventor

Michael William Grady

Date: 17/1/2000

Citizenship: United Kingdom

Residence: 74 Hall Drive Cobt

Post Office Address: Burley-in-Wharfedale, Near Ilkley, West Yorkshire LS29 7LP, United Kingdom

Inventor's Signature:
Full Name of Second Joint
Inventor, If Any

Laura Sinclair

Date: 17/01/00

Citizenship: United Kingdom

Residence: 16 Romille Street Cobt

Post Office Address: Skipton, North Yorkshire BD23 2PJ, United Kingdom

3-20
Inventor's Signature:
Full Name of Third Joint
Inventor, If Any

Paul Houston

Date: _____

Citizenship: United Kingdom
Residence: 39 Swanston Muir
Post Office Address: Edinburgh EH10 7HT, United Kingdom

CSX

(Supply similar information and signature for fourth and
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4-20
Inventor's Signature:
Full Name of Fourth Joint
Inventor, If Any

Peter John Doyle

Date: _____

Citizenship: United Kingdom
Residence: Molverley House, London Road
Post Office Address: Stapely, Nantwich, Cheshire CW5 7JS,
United Kingdom

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DOCKET NO. JJM-399

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled SULFATED POLYSACCHARIDES AND USES THEREOF IN MEDICAL TREATMENT, the specification of which

(check one) ☐ is attached hereto.

☒ was filed on March 5, 1999 as

Application Serial No. 09/254,407

and was amended on _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

Country	Application Number	Date of Filing	Priority Claimed Under 35 U.S.C. 119
Great Britain	9618958.4	September 11, 1996	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
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